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Efficacy of several potential biocontrol organisms against *Rhizoctonia solani* on potato ☆

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Abstract

Twenty-eight potential biocontrol organisms were tested for efficacy against *Rhizoctonia solani* on potato in a series of greenhouse trials. Organisms tested consisted of field isolates of *Paenibacillus polymyxa*, *Pseudomonas fluorescens*, *Penicillium* sp., *Trichoderma* sp., and *Rhizoctonia zeae*; known biocontrol isolates including *Laetisaria arvalis*, *Verticillium biguttatum*, *Cladorrhinum foecundissimum*, and *Stilbella aciculosa*; and commercial products of *Bacillus subtilis* (Kodiak), *Trichoderma virens* (SoilGard), and *T. harzianum* (RootShield). Different formulations and rates of several fungal isolates and the efficacy of combinations of effective antagonists were also investigated. None of the treatments, including a chemical control (azoxystrobin), effectively controlled stem canker and black scurf in all trials. However, *B. subtilis* GB03, *R. zeae* LRNE17E, *S. aciculosa* 112-B, and the chemical control were most effective in reducing stem canker severity (40–49% reduction) relative to the infested controls over all trials. *L. arvalis* ZH-1, *R. zeae* LRNE17E, and the chemical control reduced black scurf severity 54–60% relative to the infested control. Other treatments also significantly reduced stem canker and black scurf, however they were slightly less effective. Biocontrol amendment rate was not correlated with disease control, although the higher rates usually provided the best control. One combination of biocontrol organisms, *B. subtilis* and *T. virens*, demonstrated somewhat better control of stem canker than each organism alone, suggesting that this approach may provide improved biocontrol efficacy.

Keywords: Biological control; Potato; *Rhizoctonia solani*; Soil microbiology

1. Introduction

Rhizoctonia solani Kühn is an important fungal pathogen (Baker, 1970) that causes both stem canker and black scurf of potato (*Solanum tuberosum* L.), which lead to tuber yield reductions and losses in tuber quality. Stem canker consists of stem lesions that can reduce tuber yield by reducing the transport of nutrients throughout the plant. Black scurf is the formation of sclerotia, the long-term survival structure of the fungus, on newly formed tubers. Currently, Rhizoctonia disease is managed by cultural practices, such as crop rotation with grains, and methods that minimize prolonged contact of the plant or tubers with the pathogen, such as planting in warmer, drier conditions to promote rapid sprout emergence and promptly removing tubers from the field (Secor and Gudmestad, 1999). Chemical fungicides are often used when losses from *R. solani* are great (Parry, 1990). However, current cultural and chemical controls are not completely effective and Rhizoctonia disease remains a persistent problem.

Biological control of Rhizoctonia diseases has been demonstrated in some cases and represents an additional strategy that may provide effective and sustainable management. Biocontrol can be an effective means of control in many instances where chemical control is not available or practical (Lumsden and Papavizas, 1988). Several microbial antagonists, some of which are

☆ Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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available in commercial formulations, have shown potential for control of *R. solani* on potato or other host crops. *Trichoderma harzianum* Rifai and *Trichoderma* (*Gliocladium*) *virens* Miller, Giddens, and Foster have successfully suppressed *R. solani* in several pathosystems (Beagle-Ristaino and Papavizas, 1985; Lewis et al., 1995a, 1998; Lewis and Larkin, 1997). *Verticillium biguttatum* Gams (Velvis and Jager, 1983; Jager and Velvis, 1984, 1985, 1986), *Bacillus subtilis* (Ehrenberg) Cohn (Asaka and Shoda, 1996), *Pseudomonas fluorescens* Migula (Kloepper, 1991; Nielsen and Sørensen, 1997; Bagnasco et al., 1998), *Stilbella aciculosa* (Ellis and Everh.) Seifert (Lewis and Papavizas, 1993), *Cladorrhinum foecundissimum* Saccardo and Marchal (Lewis et al., 1995b; Lewis and Larkin, 1998), *Paenibacillus polymyxa* Prazmowski (Nielsen and Sørensen, 1997), and *Laetisaria arvalis* Burdsall (Lewis and Papavizas, 1992; Murdoch and Leach, 1993) have all shown some potential for disease control under the conditions studied. However, many of these organisms have not been studied for suppression of Rhizoctonia disease of potato and most have only been evaluated under limited conditions and have not been compared with each other in the same pathosystem.

Although the efficacy of many biocontrol organisms has been demonstrated under certain conditions, disease control is not always consistent (Larkin et al., 1998). One way to improve biocontrol is to use multiple antagonists in effective combinations (Lumsden and Papavizas, 1988; Larkin et al., 1998). The benefits of this approach include multiple mechanisms of action, synergistic effects, and wider ecological ranges of activity (Larkin et al., 1998).

The objective of this study was to determine the efficacy of several biocontrol organisms in the control of Rhizoctonia disease of potato in the greenhouse. These organisms included: field isolates of *Trichoderma* sp., *P. polymyxa*, and *P. fluorescens*; known biocontrol isolates of *L. arvalis*, *V. biguttatum*, *C. foecundissimum*, and *S. aciculosa*; and commercially available products consisting of the organisms *B. subtilis*, *T. virens*, and *T. harzianum*. In addition, the role of organisms associated with potato cropping systems in Maine and their potential biocontrol properties were investigated, including a *Penicillium* sp. that is associated with potato roots (Larkin, 2003) and *Rhizoctonia zeae*, which is associated with ryegrass rotations. The efficacy of several combinations of effective antagonists was also investigated.

2. Materials and methods

2.1. Inoculum preparation

An isolate of *R. solani* (RS31B) recovered from diseased potato roots from a field in Maine was used as the source of pathogen inoculum in all experiments. This isolate caused considerable stem canker and black scurf in preliminary greenhouse trials (Brewer and Larkin, unpublished). Fatty acid methyl ester analysis (FAME) confirmed that this isolate belonged to AG-3 (Larkin, unpublished). Inoculum was prepared by transferring four plugs of potato dextrose agar (PDA, Difco Laboratories, Inc., Sparks, MD) containing 7- to 14-day-old cultures of *R. solani* to Petri dishes filled with 20–30 g of sterile organic cracked wheat. The particle size of the cracked wheat was approximately 1 mm^3. The grain was prepared by adding 3 ml deionized water per 5 g of organic cracked wheat (Associated Buyers, Barrington, NH) and autoclaving for 60 min on two consecutive days (Papavizas and Lewis, 1986). The wheat culture of *R. solani* was incubated at room temperature (21–25 °C) for 8 days then air dried for 48 h. Viability of inoculum was confirmed by plating on PDA. Inoculum was kept in a paper bag at 4–5 °C for no more than 1 month until needed.

2.2. Soil and seed preparation

Field soil from Newport, ME, an unnamed variant of a Bangor silt loam (coarse-loamy, mixed, frigid, Typic Haplorthod), was used for all experiments. The level of native, pathogenic *R. solani* was monitored by implementing a noninfested control, which contained no added inoculum, in all experiments. The soil was sieved through a 6 × 6 mm screen and combined with sterile sand at a 3:1 w/w soil to sand ratio. Pathogen inoculum was incorporated at a rate of 4 g per kg of soil mix and incubated in the dark at room temperature for 24 h. This rate provided approximately 25 propagules of *R. solani* per gram of soil.

Potato variety 'Shepody' was used in all experiments. Shepody is susceptible to soilborne diseases, such as Rhizoctonia disease and common scab (*Streptomyces scabiei*). Seedpieces were prepared by washing in warm water, removing visible sclerotia, and treating for 2 min in 2% formaldehyde in water to prevent contamination by seedborne inoculum (Bandy et al., 1988). Seed was green-sprouted in the greenhouse for approximately 2 weeks. Seed pieces were cut to approximately 3–6 cm in length containing at least one viable sprout per piece. The seedpieces were removed from the greenhouse and allowed to suberize at room temperature for 5–7 days prior to planting.

Approximately 1200 g of pathogen-infested soil was added to each 15-cm-diameter pot and three seedpieces were arranged in the pot 2–3 cm below the soil surface. In addition, control treatments with no pathogen inoculum (a noninfested control, a sterile wheat control, and a *Penicillium* sp. control) were prepared. The wheat control consisted of 4 g of sterile cracked wheat per kilogram of noninfested soil mix to determine the effect,

if any, of the cracked wheat on Rhizoctonia disease. The *Penicillium* sp. control was used to determine the effect of this fungus, which is associated with potato roots in the field (Larkin, 2003), on the potato plant.

Azoxystrobin (Quadris, Syngenta Crop Protection, Greensboro, NC), a broad-spectrum protectant fungicide, was used as a chemical control for comparison purposes. It was prepared by applying 1 ml of a 0.1% aqueous solution around each seed piece (3 ml per pot), the manufacturer's recommended rate.

2.3. Biocontrol treatments

The biocontrol treatments, consisting of different formulations, concentrations, and combinations, were applied in several screening trials. All field isolates were acquired from soil at potato fields in Newport or Presque Isle, ME.

Bacterial antagonists included *B. subtilis* GB03, the active ingredient of Kodiak (Gustafson, Dallas, TX), five field isolates of *P. polymyxa*, and three field isolates of *P. fluorescens*. Treatments consisted of aqueous solutions prepared from 3-day-old cultures of the bacteria grown on tryptic soy agar (Difco Laboratories, Inc.). Solutions were adjusted to transmittance values of 50% ($\pm$2%) at 590 nm and concentrations were determined by plating on tryptic soy agar. Suspensions contained concentrations of 10^7–10^8 cfu/ml for all bacterial preparations except *P. polymyxa* which contained 10^6 cfu/ml. Thirty milliliters of each suspension were added to 1200 g of field soil-sand mix and incorporated prior to planting.

SoilGard (Olympic Horticultural Products, Mainland, PA), a commercial formulation of *T. virens* GL-21, was added at rates of 2.6 g per pot and 1.3 g per pot (0.2% and 0.1% incorporation rate [w/w], respectively). The RootShield drench (BioWorks, Geneva, NY), containing *T. harzianum* T-22, was added to the soil as directed by the manufacturer at a rate of 240 ml of drench (0.59 g/L of water) per pot of soil.

Aqueous spore suspensions of the active ingredients of SoilGard and RootShield, several *Trichoderma* field isolates, *V. biguttatum* M73 (ATCC #90587), and three *Penicillium* sp. field isolates were prepared from active, sporulating cultures growing on PDA (8–14 day old cultures for *Trichoderma* isolates and 14–21 days old for *Verticillium* and *Penicillium* isolates). Spore concentrations were adjusted to 10^7 conidia per milliliter for *Trichoderma* isolates, and 10^6 conidia per milliliter for *V. bigutatum* and *Penicillium* sp. *T. virens* was also added at rates of 10^6 conidia per milliliter and 10^5 conidia per milliliter to determine if biocontrol was also effective at lower doses. Thirty milliliters of each suspension were added to each pot and incorporated prior to planting.

R. zeae field isolates RZLRNE17E and RZRF27I, *R. zeae* 504 (obtained from Roseann Leiner, Fairbanks, AK), *L. arvalis* LA-1 (ATCC #52088), and *L. arvalis* ZH-1 (ATCC #62715) were also screened. These nonspore-forming fungal treatments were prepared on cracked wheat as described above for the pathogen inoculum except the incubation time was decreased to 5 days. Three grams of colonized wheat was added to soil near the seedpiece to simulate an in-furrow application. One and a half grams of *L. arvalis* ZH-1 amendment were also added as a separate treatment to determine the most effective rate.

C. foecundissimum C91 (ATCC #62373), *S. aciculosa* 112B-1 (ATCC #90820), and an additional *L. arvalis* LA-1 treatment were prepared by adding a 14-day-old, 10-day-old, or 7-day-old, respectively, Petri dish of PDA covered with the culture to 150 ml sterile water. The solution was mixed vigorously on a stir plate for 30 min and 30 ml was added to each pot and incorporated prior to planting.

Plants were grown in the greenhouse for 3–4 weeks until the noninfested and wheat controls had fully emerged. Plants were harvested, washed, and rated for stem canker on a scale of 0–5 as follows: 0 = no disease symptoms; 1 = brown discoloration of stems; 2 = cankers covering $<25\%$ of the stem circumference; 3 = 25–75% stems covered by cankers; 4 = 75% coverage by stem cankers; and 5 = stem completely nipped off or death of the plant. When two or more shoots emerged from one seed piece the average rating of all shoots was recorded. Incidence of stem canker was measured as the percentage of plants with a severity rating of 2 or greater. After harvest, newly formed sclerotia were visible on the seed pieces, therefore, black scurf was also assessed on a scale of 0–5 as follows: 0 = no visible sclerotia; 1 = sclerotia covering 1% of the skin-covered tuber surface; 2 = 2–5% covered; 3 = 5–10% covered; 4 = 10–15% covered; and 5 = $>15\%$ tuber covered by sclerotia. Incidence of black scurf was calculated as the percent of tubers with a severity rating of 1 or greater. Differences in shoot size among treatments were also detected at harvest, so shoots were either weighed or rated for size. In the first trial, weight measurements were taken for roots and shoots of each plant. In subsequent trials (trials 2–4), ratings of 0–3 (0 = no growth, 1 = shoot height <2 cm, 2 = shoot height of 2–5 cm, and 3 = shoot height >5 cm) were used.

2.4. Statistical analysis

All trials used a randomized complete block design. There were three replications of each treatment in the first experiment and four replications per treatment in trials 2–4. Each replicate consisted of one pot containing three potato seedpieces. Data from each trial was

analyzed separately by analysis of variance using SAS (ver. 7, SAS Institute, Cary, NC). In addition, data representing percent disease suppression for each treatment were combined for multiple trials and analyzed to compare overall efficacy rates. Mean separation was accomplished using Fisher's least significant difference (LSD) at $P = 0.05$.

3. Results

3.1. Efficacy of biocontrol organisms

There were significant treatment by trial interactions for severity and incidence of stem canker and black scurf. Therefore, each trial is presented separately. However, there were trends in the efficacy of many of the treatments. The level of native, pathogenic *R. solani*, which was measured by the noninfested control, varied among the four trials. Field soil was collected from the same location for all trials, but varying environmental conditions at the time of collection may explain the variability across all trials and the elevated level of disease pressure in trial 4.

In the first trial of biocontrol organisms, 26 different isolates and three controls were tested. Treatments that reduced the incidence (50–89%) and severity of stem canker (35–63%) relative to the infested control included the chemical control, *T. virens* GL-21 (spore suspension), *Trichoderma* TW, *Trichoderma* T2, *T. harzianum* T-22 (spore suspension), *R. zeae* RF27I, *P. fluorescens* 3, *P. polymyxa* 2, *P. polymyxa* 3, and *P. polymyxa* 5 (Table 1). The *Trichoderma* TY treatment also reduced stem canker severity by 39%. Treatments of *L. arvalis* ZH-1, *Penicillium* YG 1, *Penicillium* YG 2, *Penicillium* YG 3, *R. zeae* 504, all *Trichoderma* isolates except *Trichoderma* TY, *V. biguttatum* M73, and the chemical control each reduced black scurf severity (46–100%) relative to the infested control. Treatments that provided some control for both stem canker and black scurf in trial 1 included *T. harzianum* T-22 (spore suspension), *T. virens* GL-21 (spore suspension), *Trichoderma* T2, and *Trichoderma* TW. Based on the results of this first trial, the number of biocontrol organisms was reduced by selecting no more than two isolates per species to be used in subsequent tests. Several new organisms were also added to later trials.

In subsequent trials, all isolates tested reduced incidence or severity of stem canker in at least one of the three tests, except *R. zeae* RF27I and *Trichoderma* T2 (Table 2). However, no treatment, including the chemical control, reduced both incidence and severity of stem canker in all tests. The *B. subtilis* treatment reduced stem canker severity in all three tests, but incidence was reduced in only one test. The *P. polymyxa* 3 treatment reduced severity in two of three trials and incidence in one trial. Treatments of *R. zeae* LRNE17E and *S. aciculosa* reduced incidence and severity of stem canker in two trials. The *Penicillium* YG 2 treatment reduced severity of stem canker in two trials and the *L. arvalis* ZH-1 treatment reduced incidence in two trials.

Most isolates tested, with the exception of *Penicillium* YG 3, *R. zeae* RF27I, *Trichoderma* T2, and *T. harzianum* (spores suspension), also reduced the incidence and severity of black scurf in at least one of three subsequent trials (Table 3). None of the treatments reduced incidence and severity of black scurf across all trials. The *R. zeae* LRNE17E and *L. arvalis* ZH-1 treatments reduced severity in all trials and the *R. zeae* LRNE17E treatment reduced incidence in one of three trials. The chemical control, *Penicillium* YG 2, *T. virens* GL-21 (spore suspension), *P. polymyxa* 2, and *P. polymyxa* 3 treatments reduced black scurf severity in two of three trials. The chemical control and the *Penicillium* YG 2 treatment reduced incidence in two of three and one of three trials, respectively.

When averaged over all trials, *B. subtilis*, *R. zeae LRNE17E*, *S. aciculosa*, and the chemical control treatments were most successful at reducing stem canker severity with reductions of 40–49% relative to the infested control (Fig. 1A). *P. fluorescens* 3, *P. polymyxa* 3, *P. polymyxa* 2, *C. foecundissimum*, *L. arvalis* ZH-1, *Penicillium* YG 2, *Penicillium* YG 4, *T. virens* (spore suspension and formulation), *T. harzianum* (spore suspension), and *Trichoderma* T2 also reduced stem canker severity (29–39%). Treatments that reduced black scurf severity most effectively across all trials (54–60%) included *L. arvalis* ZH-1, *R. zeae* LRNE17E, and the chemical control (Fig. 1B). Other treatments that significantly reduced black scurf (38–44%) included *B. subtilis*, *P. fluorescens* 3, *Penicillium* YG 2, *V. biguttatum*, and *T. virens* (spore suspension and formulation).

Several treatments showed some control of both aspects of Rhizoctonia disease when reductions in stem canker severity and black scurf severity were averaged across all trials. The most notable treatment was *R. zeae* LRNE17E, which reduced stem canker 49% and black scurf 58%. Other treatments that controlled both stem canker and black scurf included *B. subtilis*, *P. fluorescens* 3, *L. arvalis* ZH-1, *Penicillium* YG 2, *T. virens* (spore suspension and formulation) and the chemical control (Fig. 1).

The size of potato shoots also varied among treatments when means were averaged across trials 2–4. Several treatments reduced plant size when compared to the noninfested control (Fig. 2). As expected, the infested control was one of these treatments. However, *P. polymyxa* 2, *P. fluorescens* 2, *C. foecundissimum*, *V. biguttatum*, and *T. harzianum* (spore suspension) treatments also reduced shoot size. Some treatments tested in trial 1 also reduced shoot size. These included

Table 1
Effect of potential biocontrol organisms on Rhizoctonia stem canker and black scurf of potato for trial 1

Treatment	Stem canker			Black scurf	
	Incidence[a] (%)	Severity[b]	Reduction[c] (%)	Severity[d]	Reduction[e] (%)
B. subtilis GB03	61.1	1.9	30.0	3.0	18.0
L. arvalis ZH-1	55.6	1.9	29.4	1.7*	54.7*
L. arvalis LA-1	77.8	2.7	0.1	3.3	9.0
P. polymyxa 1+[f]	72.2	2.1	20.6	3.7	0.0
P. polymyxa 2+	44.4*[g]	1.6*	42.0*	2.7	27.7
P. polymyxa 3+	44.4*	1.6*	42.0*	3.3	9.0
P. polymyxa 4+	66.7	1.9	29.4	3.0	18.0
P. polymyxa 5+	50.0*	1.8*	34.8*	3.0	18.3
Penicillium sp. YG 1+	72.2	2.3	15.8	1.0*	72.7*
Penicillium sp. YG 2+	77.8	1.9	29.9	1.0*	73.0*
Penicillium sp. YG 3+	100.0	2.6	2.6	1.3*	64.0*
P. fluorescens 1+	77.8	2.1	20.6	3.0	18.3
P. fluorescens 2+	77.8	2.0	25.1	2.3	36.7
P. fluorescens 3+	33.3*	1.7*	37.8*	3.3	18.3
R. circinata W616	100.0	2.8	−2.9	2.3	36.7
R. circinata W630	88.9	2.3	12.8	2.3	36.3
R. zeae LRNE17E+	55.5	1.9	30.0	2.3	36.3
R. zeae RF27I+	50.0*	1.5*	44.0*	3.0	18.0
R. zeae RZ504	88.9	2.1	21.0	1.7*	54.7*
T. harzianum T-22	27.8*	1.3*	52.1*	2.0*	46.0*
Trichoderma sp. T2+	33.3*	1.5*	44.1*	0.0*	100.0*
Trichoderma sp. TN2RA+	66.7	1.9	29.3	1.3*	64.0*
Trichoderma sp. TW+	38.9*	1.6*	41.4*	1.3*	64.0*
Trichoderma sp. TY+	66.7	1.6*	39.3*	2.3	36.7
T. virens GL-21	33.3*	1.3*	50.3*	0.7*	82.0*
V. biguttatum M73	66.7	2.3	12.8	0.7*	82.0*
Infested control	100.0	2.7	0.0	3.7	0.0
Noninfested control	0.0*	0.2*	91.8*	0.0*	100.0*
Chemical control (azoxystrobin)	11.1*	1.0*	62.9*	2.0*	46.0*
LSD ($P = 0.05$)	46.2	0.9	34.4	1.4	37.2

[a]Incidence of plants with obvious lesions (severity rating of 2 or higher).
[b]Severity ratings for stem canker were on a scale of 0–5 (0 = no symptoms; 1 = brown discoloration; 2 = cankers covering<25% of the stem circumference; 3 = 25–75% coverage by cankers; 4 = 75% coverage by cankers; and 5 = stem completely nipped off or death of the plant).
[c]Percent reduction in stem canker severity relative to the infested control.
[d]Severity ratings were as follows: 0 = no visible sclerotia; 1 = sclerotia covering 1% of the skin-covered tuber surface; 2 = 2–5% covered; 3 = 5–10% covered; 4 = 10–15% covered; and 5 = >15% tuber covered by sclerotia.
[e]Percent reduction in black scurf severity relative to the infested control.
[f]Isolate names followed by a plus (+) sign are soil isolates isolated from a potato field in Maine.
[g]Values followed by an asterisk are significantly different from the infested control according to Fisher's LSD ($P = 0.05$).

P. fluorescens 1, *Penicillium* YG 1, *Trichoderma* TN2RA, and *Trichoderma* TW. Whether these treatments reduced shoot size due to delayed emergence, deleterious effects on the plants themselves, or because the treatments did not effectively suppress *R. solani* could not be determined from these tests. However, none of the treatments reduced plant size relative to the infested control.

The addition of sterile wheat or *Penicillium* YG 2 to noninfested soil did not affect disease levels or plant size compared to the noninfested control (Table 4). Although not significant, the *Penicillium* YG 2 treatment tended to have slightly higher incidence and severity of both stem canker and black scurf than the other noninfested controls. Perhaps the presence of this fungus influences populations or activity of native *Rhizoctonia* in the soil in some way.

Two different formulations of *L. arvalis* LA-1 were evaluated in one trial. The formulations, a blended culture of the fungus on PDA and the fungus grown on cracked wheat, were similar, with neither formulation reducing stem canker or black scurf. Reductions in stem canker severity were 0.1% and −2.8% for the cracked wheat and PDA formulation, respectively. Reductions in black scurf severity were 9.0% for the cracked wheat and 18.3% for the PDA formulation.

The initial 3 g per pot rate of *L. arvalis* ZH-1 was compared with a reduced rate of 1.5 g per pot in one

Table 2
Incidence and severity of Rhizoctonia stem canker on potato shoots as affected by biocontrol treatments for trials 2, 3, and 4

Treatment	Trial 2		Trial 3		Trial 4	
	Incidence[a] (%)	Severity[b]	Incidence (%)	Severity	Incidence (%)	Severity
B. subtilis GB03	33.3*[c]	1.6*	50.0	1.6*	54.2	1.7*
C. foecundissiumum C91	66.7	2.4	50.0	1.9*	—	—
L. arvalis ZH-1	41.7*	2.3	33.3*	1.3*	66.7	2.1
L. arvalis LA-1	—[d]	—	41.7*	1.7*	—	—
P. polymyxa 2^{+}[e]	83.3	3.6	33.3*	1.2*	58.4	1.9
P. polymyxa 3^{+}	75.0	2.9	33.4*	1.4*	50.0	1.8*
Penicillium sp. YG 2^{+}	66.7	1.8*	66.7	2.2	66.7	1.8*
Penicillium sp. YG 3^{+}	—	—	33.3*	1.1*	83.4	2.2
P. fluorescens 2^{+}	66.7	2.9	61.1	1.9*	—	—
P. fluorescens 3^{+}	75.0	2.6	20.8	1.1*	41.7	1.9
R. zeae LRNE17E^{+}	33.3*	1.1*	16.7*	0.8*	66.7	1.9
R. zeae RF27I^{+}	—	—	—	—	91.7	2.8
S. aciculosa 112B-1	37.5*	1.8*	58.4	1.6*	—	—
T. harzianum T-22	—	—	—	—	50.0	1.8
T. harzianum T-22 (form.)	—	—	54.2	1.7*	70.8	2.4
Trichoderma sp. T2^{+}	—	—	—	—	50.0	1.9
T. virens GL-21	50.0*	2.4	62.5	2.0	66.7	2.0
T. virens GL-21 (form.)	58.4*	2.3	16.7*	1.2*	70.9	1.8
V. biguttatum M73	66.7	3.0	58.3	1.7*	50.0	1.8
Infested control	100.0	3.5	91.7	2.8	83.4	2.5
Noninfested control	8.3*	0.3*	0.0*	0.0*	29.2*	1.0*
Chemical control (azoxystrobin)	33.3*	1.7*	33.4*	0.9*	66.7	2.3
LSD ($P = 0.05$)	41.6	1.4	42.7	0.9	42.6	0.7

[a]Incidence of plants with obvious lesions (severity rating of 2 or higher).
[b]Severity ratings for stem canker were on a scale of 0–5 (0 = no symptoms; 1 = brown discoloration; 2 = cankers covering<25% of the stem circumference; 3 = 25–75% coverage by cankers; 4 = 75% coverage by cankers; and 5 = stem completely nipped off or death of the plant).
[c]Values followed by an asterisk are significantly different from the infested control according to Fisher's LSD ($P = 0.05$).
[d]Treatment was not tested in this trial.
[e]Isolate names followed by a plus (+) sign are soil isolates isolated from a potato field in Maine.

trial. The reduced rate treatment resulted in higher incidence (66.7%) and less reduction in severity (22.2%) of stem canker than the initial rate (41.7% incidence and 34.3% reduction in severity). The initial rate treatment significantly lowered the incidence of stem canker, whereas the reduced rate did not. Both treatments performed similarly for control of black scurf. Incidence was 50% for the initial rate treatment and 58% for the reduced rate treatment. The reduction in black scurf severity was 66.8% for both treatments.

Different formulations, as a spore suspension and the commercial product, of *T. virens* GL-21 and *T. harzianum* T-22 were also evaluated. When averaged over all trials, the spore suspension treatment of *T. harzianum* T-22 reduced stem canker, whereas the commercial product treatment did not (Fig. 1A). Neither treatment reduced black scurf (Fig. 1B). However, the commercial product treatment did reduce stem canker severity (Tables 1 and 2) and black scurf severity (Table 3) in one of two trials, and incidence and severity of black scurf were also reduced in one of two tests for the spore suspension. When reductions were averaged across all trials for both formulations of *T. virens* GL-21, both the spore suspension and commercial product significantly reduced both stem canker (Fig. 1A) and black scurf (Fig. 1B). In individual trials, the commercial product reduced stem canker severity in two of three trials and incidence in one trial, whereas the spore suspension treatment reduced incidence of stem canker in only one of three trials (Table 2). However, the spore suspension treatment reduced black scurf severity in two of three trials and the commercial product treatment reduced incidence and severity in one of three trials (Table 3).

In one trial, different rates of the spore suspension and commercial product of *T. virens* GL-21 were compared. All treatments reduced the incidence of stem canker relative to the infested control and had similar incidence values (Table 5). For stem canker severity, neither the commercial formulation nor spore suspension reduced severity. Only the commercial formulation treatment at the higher rate reduced incidence of black scurf. However, all treatments, with the exception of the spore suspension at the lowest rate, reduced black scurf

Table 3
Influence of biocontrol agents on black scurf incidence and severity for trials 2, 3, and 4

Treatment	Trial 2		Trial 3		Trial 4	
	Incidence (%)	Severity[a]	Incidence (%)	Severity	Incidence (%)	Severity
B. subtilis GB03	50.0	0.6*[b]	79.2	1.9	54.2*	1.2
C. foecundissiumum C91	66.7	0.8*	100.0	2.3	—	—
L. arvalis ZH-1	50.0	0.6*	75.0	1.0*	75.0	0.8*
L. arvalis LA-1	—[c]	—	83.3	1.5*	—	—
P. polymyxa 2+[d]	75.0	0.8*	91.7	1.5*	75.0	1.7
P. polymyxa 3+	66.7	0.9*	100.0	1.6*	91.7	1.4
Penicillium sp. YG 2+	58.3	0.7*	100.0	2.4	50.0*	0.7*
Penicillium sp. YG 3+	—	—	100.0	2.3	66.7	1.5
P. fluorescens 2+	75.0	0.9*	88.9	2.0	—	—
P. fluorescens 3+	66.7	0.8*	100.0	1.4*	58.4	1.3
R. zeae LRNE17E+	75.0	0.8*	91.7	1.3*	33.3*	0.5*
R. zeae RF27I+	—	—	—	—	100.0	1.8
S. aciculosa 112B-1	75.0	1.1*	100.0	2.3	—	—
T. harzianum T-22	—	—	—	—	91.7	1.4
T. harzianum T-22 (form.)	—	—	100.0	1.7*	79.2	1.4
Trichoderma sp. T2+	—	—	—	—	100.0	2.1
T. virens GL-21	75.0	1.1*	87.5	2.1	66.7	0.9*
T. virens GL-21 (form.)	33.3*	0.3*	100.0	1.9	70.9	1.6
V. biguttatum M73	58.3	0.8*	87.5	1.8	75.0	1.4
Infested control	91.7	1.8	100.0	2.4	100.0	1.8
Noninfested control	16.7*	0.2*	0.0*	0.0*	8.3*	0.1*
Chemical control (azoxystrobin)	33.3*	0.4*	75.0*	1.0*	83.4	1.4
LSD ($P = 0.05$)	42.3	0.6	23.5	0.7	42.4	0.7

[a]Severity ratings were as follows: 0 = no visible sclerotia; 1 = sclerotia covering 1% of the skin-covered tuber surface; 2 = 2–5% covered; 3 = 5–10% covered; 4 = 10–15% covered; and 5 = >15% tuber covered by sclerotia.
[b]Values followed by an asterisk are significantly different from the infested control according to Fisher's LSD ($P = 0.05$).
[c]Treatment was not tested in this trial.
[d]Isolate names followed by a plus (+) sign are soil isolates isolated from a potato field in Maine.

severity. The higher rate of the commercial formulation and the 10^7 rate of the spore suspension were most effective in reducing black scurf.

3.2. Biocontrol combinations

Some combinations of biocontrol organisms effectively reduced Rhizoctonia disease in one trial. The combination of *B. subtilis* GB03 and *T. virens* GL-21 was the only treatment to reduce the incidence of stem canker (Table 6). This combination and the *L. arvalis* ZH-1/*T. virens* GL-21 combination reduced stem canker severity. *B. subtilis* GB03 alone reduced stem canker severity (30.9%), but the *B. subtilis* GB03/*T. virens* GL-21 combination was slightly more effective (46.7%). The combined treatments of *Penicillium* YG 2/*R. zeae* LRNE17E, *Penicillium* YG 2/*L. arvalis* ZH-1, and *L. arvalis* ZH-1/*T. virens* GL-21 all reduced incidence of black scurf. As individual treatments, *Penicillium* YG 2 and *R. zeae* LRNE17E also had lower incidence of black scurf. *R. zeae* LRNE17E was more effective alone (33.3%) at reducing black scurf incidence than in combination with *Penicillium* YG 2. All combinations that reduced black scurf incidence also reduced black scurf severity. The *L. arvalis* ZH-1/*V. biguttatum* M73 combination also reduced black scurf severity. *Penicillium* YG 2 (63.8%), *R. zeae* LRNE17E (72.8%), and *L. arvalis* ZH-1 (54.9%) also reduced black scurf severity alone. The *Penicillium* YG 2/*L. arvalis* ZH-1 combination tended to be most effective at reducing black scurf, and was slightly more effective than either organism alone. However, only one combination, *L. arvalis* ZH-1/*T. virens* GL-21 controlled both stem canker and black scurf in this test. Thus, little improvement in controlling both aspects of Rhizoctonia disease was observed with the combinations in this test.

4. Discussion

Biological control of *R. solani* on various crops has much potential for disease management, though there are several problems with practical implementation (Cook and Baker, 1983; Velvis and Jager, 1983; Murdoch and Leach, 1993; Lewis et al., 1995a, b; Lewis and Larkin, 1998). Numerous organisms demonstrated biocontrol capability against *R. solani* in the present study, but there was also much variability in control

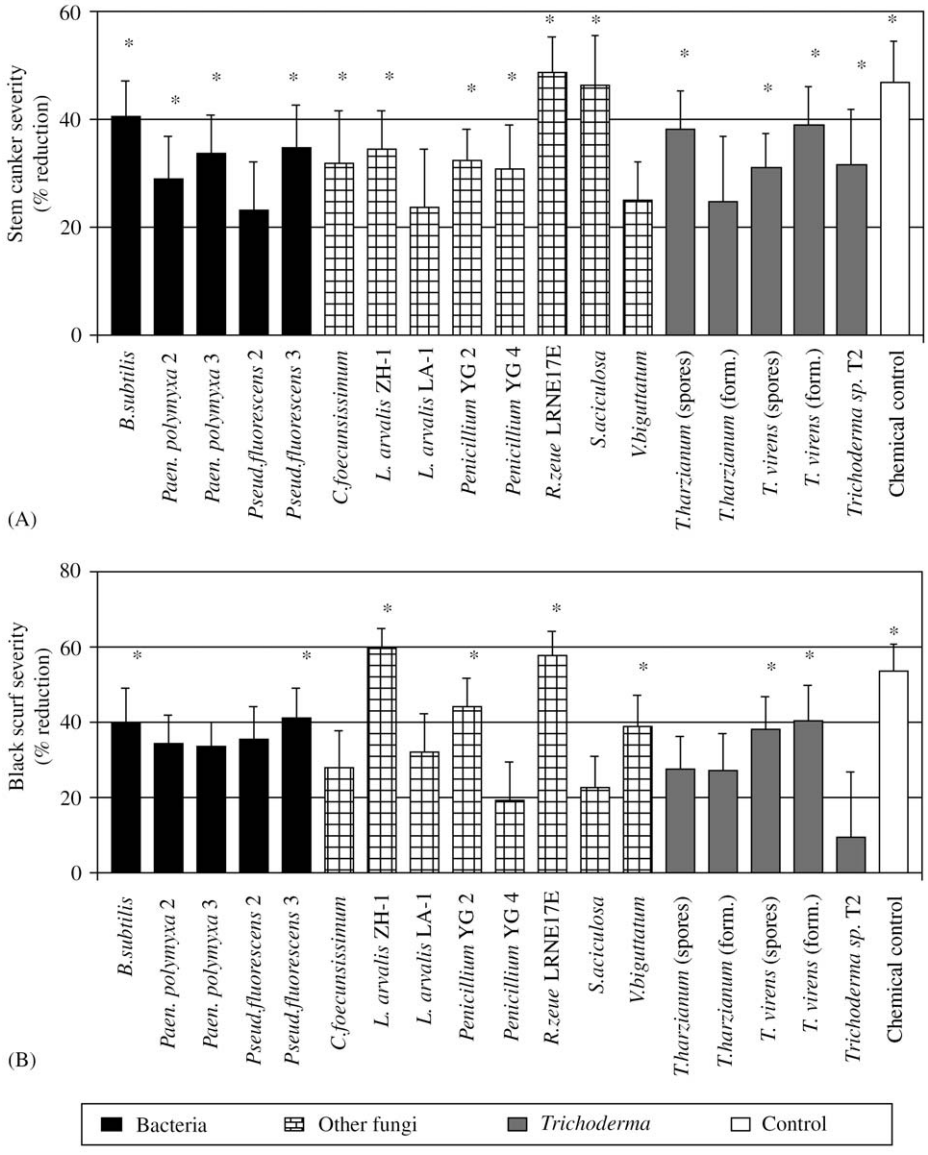

Fig. 1. Mean reductions in (A) Rhizoctonia stem canker severity and (B) black scurf severity of potato by select biocontrol treatments and a chemical control relative to the infested control across all trials. *Pseud.* = *Pseudomonas* and *Paen.* = *Paenibacillus*. '*' Indicates a significant reduction relative to the infested control. LSD = 28.5 (stem canker) and 36.8 (black scurf). Disease severity in the infested control averaged 2.86 and 2.13 (0 to 5 scale) for stem canker and black scurf, respectively.

from one trial to the next. Efficacy of organisms against stem canker or black scurf, two important components of Rhizoctonia disease that reduce tuber yield and quality, was not consistent among trials. Although most organisms tested significantly reduced stem canker or black scurf in at least one greenhouse trial, no treatment reduced both incidence and severity of either stem canker or black scurf in all trials. Biocontrol agents also varied in their abilities to suppress both disease components. The most consistently effective treatments for control of stem canker were not necessarily the most effective for control of black scurf. Over all trials, *B. subtilis* GB03, *R. zeae* LRNE17E, *S. aciculosa* 112-B, and the chemical control were most effective at reducing stem canker severity (40–49%) and *L. arvalis* ZH-1, *R. zeae* LRNE 17E, and the chemical control were most effective at reducing black scurf severity (54–60%). Some biocontrol treatments reduced disease relative to the infested control as well as, or better than, the chemical control.

B. subtilis GB03 was among the most consistent and effective biocontrol treatments for control of stem canker. The commercial product of this isolate also reduced incidence and severity of stem canker (Larkin, 2001, 2002) and scurf severity (Larkin, 2002) on potato caused by *R. solani* in field studies. In the present study, results were also consistent with previous studies on *L. arvalis*, which can reduce population levels of *R. solani*

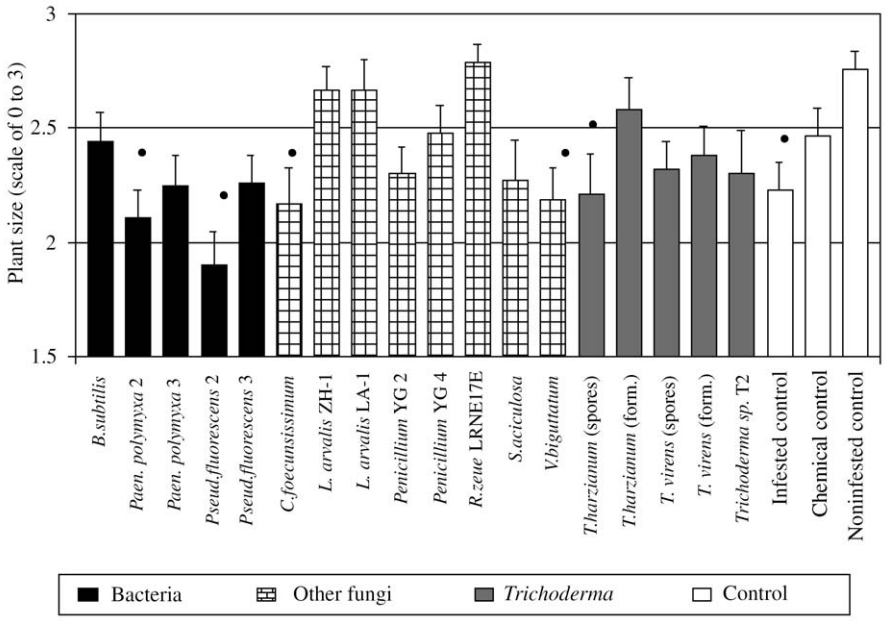

Fig. 2. Mean shoot size as affected by select biocontrol treatments across all trials. *Pseud* = *Pseudomonas* and *Paen.* = *Paenibacillus*. LSD = 0.53. '●' Indicates a significantly reduced plant size relative to the noninfested control.

Table 4
Comparison of noninfested control, wheat control, and *Penicillium* sp. control for Rhizoctonia stem canker, black scurf, and shoot size of potato across all trials

Treatment	Stem canker		Black scurf		Shoot size[d]
	Incidence[a] (%)	Severity[b]	Incidence (%)	Severity[c]	
Noninfested control	10.0	0.4	8.3	0.1	2.76
Wheat control	15.6	0.6	5.6	0.1	2.79
Penicillium YG 2 control	16.7	0.7	16.7	0.3	2.70
LSD ($P = 0.05$)	36.11	0.8	31.02	0.8	0.53

[a]Incidence of plants with obvious lesions (severity rating of 2 or higher).
[b]Severity ratings for stem canker were on a scale of 0–5 (0 = no symptoms; 1 = brown discoloration; 2 = cankers covering < 25% of the stem circumference; 3 = 25–75% coverage by cankers; 4 = 75% coverage by cankers; and 5 = stem completely nipped off or death of the plant).
[c]Severity ratings were as follows: 0 = no visible sclerotia; 1 = sclerotia covering 1% of the skin-covered tuber surface; 2 = 2–5% covered; 3 = 5–10% covered; 4 = 10–15% covered; and 5 = > 15% tuber covered by sclerotia.
[d]Shoot size was rated on a scale of 0–3 as follows: 0 = no growth; 1 = shoot height < 2 cm; 2 = shoot height of 2–5 cm; and 3 = shoot height > 5 cm.

(Allen et al., 1985; Larsen et al., 1985; Murdoch and Leach, 1993), as well as stem canker, stolon canker, and black scurf of potato (Murdoch and Leach, 1993).

V. bigutattum has been studied extensively for suppression of *R. solani* on potato (Velvis and Jager, 1983; Jager and Velvis, 1984, 1985; Jager et al., 1991; Wicks et al., 1995) and has been previously reported to suppress *R. solani* from both tuberborne (Velvis and Jager, 1983) and soilborne (Jager and Velvis, 1984) inoculum sources. Although most success with *V. biguttatum* has been with control of black scurf, it has also successfully reduced stem canker (Jager and Velvis, 1984). Previous studies demonstrated that *V. biguttatum* was more effective against *R. solani* than the biocontrol organisms *Gliocladium* and *Trichoderma* (Velvis and Jager, 1983). In the present study, treatment with *V. biguttatum* did not reduce stem canker. It was somewhat effective at reducing black scurf, although reductions were less than with some other more effective organisms. The efficacy of *V. biguttatum* varies based on the type of soil used with it being least effective in sandy soils due to high population levels of *R. solani* (Jager and Velvis, 1985). The soil used in the present study was a sandy soil, which may account for the low success of *V. biguttatum* demonstrated.

S. aciculosa reduced damping off caused by *R. solani* on cotton, sugarbeet, and radish (Lewis and Papavizas, 1993). Nielsen and Sørensen (1997) determined that

Table 5
Comparison of *Trichoderma virens* GL-21 rates and formulations on Rhizoctonia stem canker and black scurf of potato

Treatment	Stem canker			Black scruf		
	Incidence[a] (%)	Severity[b]	Reduction[c] (%)	Incidence (%)	Severity[d]	Reduction[e] (%)
Spore suspension (conidia/pot)						
T. virens GL-21 (3×10^8)	50.0*[f]	2.4	29.6	75.0	1.1*	38.3*
T. virens GL-21 (3×10^7)	54.2*	2.1	39.0	50.0	0.7*	62.0*
T. virens GL-21 (3×10^6)	58.4*	2.9	12.4	75.0	1.3	28.8
Commercial formulation						
T. virens GL-21 (2.6 g/pot)	58.4*	2.3	31.8	33.3*	0.3*	81.0*
T. virens GL-21 (1.3 g/pot)	54.2*	2.6	23.1	66.7	0.8*	52.5*
Infested control	100.0	3.5	0.0	91.7	1.8	0.3
Noninfested control	8.3*	0.3*	90.3*	16.7*	0.2*	90.5*
Chemical control	33.3*	1.7*	51.3*	33.3*	0.4*	76.3*
LSD ($P = 0.05$)	41.6	1.4	46.8	42.3	0.6	32.1

[a]Incidence of plants with obvious lesions (severity rating of 2 or higher).
[b]Severity ratings for stem canker were on a scale of 0–5 (0 = no symptoms; 1 = brown discoloration; 2 = cankers covering <25% of the stem circumference; 3 = 25–75% coverage by cankers; 4 = 75% coverage by cankers; and 5 = stem completely nipped off or death of the plant).
[c]Percent reduction in stem canker severity relative to the infested control.
[d]Severity ratings were as follows: 0 = no visible sclerotia; 1 = sclerotia covering 1% of the skin-covered tuber surface; 2 = 2–5% covered; 3 = 5–10% covered; 4 = 10–15% covered; and 5 = >15% tuber covered by sclerotia.
[e]Percent reduction in black scurf severity relative to the infested control.
[f]Values followed by an asterisk are significantly different from the infested control according to Fisher's LSD ($P = 0.05$).

Table 6
Efficacy of biocontrol combinations against Rhizoctonia disease of potato in a single, preliminary study

Treatments	Stem canker			Black scurf		
	Incidence[a] (%)	Severity[b]	Reduction[c] (%)	Incidence (%)	Severity[d]	Reduction[e] (%)
L. arvalis ZH-1/*B. subtilis* GB03	83.4	2.5	0.0	66.7	1.2	36.7
Penicillium YG2/*R. zeae* LRNE17E	75.0	2.2	13.3	41.7*	0.5*	72.9*
Penicillium YG2/*L. arvalis* ZH-1	87.5	2.5	1.8	25.0*	0.3*	85.3*
B. subtilis GB03/*T. virens* GL-21	33.3*[f]	1.3*	46.7*	75.0	1.2	36.6
R. zeae LRNE17E/*T. virens* GL-21	91.7	2.5	1.8	100.0	2.0	−8.6
B. subtilis GB03/*V. biguttatum* M73	58.3	2.1	16.7	75.0	1.2	36.7
L. arvalis ZH-1/*T. virens* GL-21	58.3	1.6*	36.7*	53.4*	1.0*	45.7*
L. arvalis ZH-1/*V. biguttatum* M73	83.4	2.6	−3.3	58.3	0.7*	63.9*
Infested control	83.4	2.5	0.0	100.0	1.8	0.0
Noninfested control	29.2*	1.0*	60.0*	8.3*	0.1*	95.1*
Chemical control	66.7	2.3	9.1	83.4	1.4	25.9
LSD ($P = 0.05$)	42.6	0.7	29.5	42.4	0.8	41.1

[a]Incidence of plants with obvious lesions (severity of 2 or higher).
[b]Severity ratings for stem canker were on a scale of 0–5 (0 = no symptoms; 1 = brown discoloration; 2 = cankers covering <25% of the stem circumference; 3 = 25–75% coverage by cankers; 4 = 75% coverage by cankers; and 5 = stem completely nipped off or death of the plant).
[c]Percent reduction in stem canker severity relative to the infested control.
[d]Severity ratings were as follows: 0 = no visible sclerotia; 1 = sclerotia covering 1% of the skin-covered tuber surface; 2 = 2–5% covered; 3 = 5–10% covered; 4 = 10–15% covered; and 5 ⩾ 15% tuber covered by sclerotia.
[e]Percent reduction in black scurf severity relative to the infested control.
[f]Values followed by an asterisk are significantly different from the infested control according to Fisher's LSD ($P = 0.05$).

strains of *P. polymyxa* inhibited *R. solani* in vitro and produced cell wall-degrading enzymes. *S. aciculosa* and three of five strains of *P. polymyxa* tested in the present study reduced stem canker. This is the first report to suggest that *S. aciculosa* and *P. polymyxa* can reduce stem canker on potato caused by *R. solani*.

The present study also details the first account of *R. zeae* and a potato root associated *Penicillium* sp. as potential biocontrols of *R. solani*. *R. zeae* is a known pathogen of turfgrasses (Martin and Lucas, 1983; Burpee and Martin, 1992). However, isolates tested in the present study did not cause disease on barley or

ryegrass (Brewer and Larkin, unpublished). Some isolates of *R. zeae*, including those tested in the present study, have produced stem lesions on potato plants, although to a much lesser degree than *R. solani* (Carling and Leiner, 1990; Brewer and Larkin, unpublished). Surprisingly, *R. zeae* LRNE17E was the most effective treatment for reducing both stem canker and black scurf relative to the infested control. The mechanism of action was not determined, but perhaps it is competition. *R. zeae* may colonize roots more effectively and out compete *R. solani*, and as a weak pathogen it may prevent *R. solani* from causing more severe disease. *R. zeae* demonstrates potential as a biocontrol organism but additional isolates need to be screened to find an isolate that is an effective antagonist, but does not cause damage or disease to important crops.

Penicillium sp. are frequently found when soil cropped to potato or potato roots are plated on culture media (Larkin, 2003). Isolates of this organism demonstrated some control of both stem canker and black scurf. This fungus may serve a role in protection of the roots and newly formed tubers from root pathogens such as *R. solani*. It did not, however, demonstrate in vitro antagonism towards *R. solani* (Brewer and Larkin, unpublished).

A treatment that suppresses both stem canker and black scurf would be best as a biocontrol strategy. Several treatments demonstrated potential to suppress both aspects in the present study. Also, combinations of organisms that provide enhanced control of both disease symptoms would be beneficial. Some combinations provided better control of stem canker, but none suppressed black scurf better than individual biocontrol organisms. Enhanced control of black scurf or stem canker of potato may also be provided by combining azoxystrobin with effective bacterial biocontrol organisms.

The formation of black scurf on the seed pieces was assessed in the present study after differences in severity were detected among the various treatments upon sprout harvest. Minimizing tuberborne inoculum is crucial in suppression of Rhizoctonia disease (Frank and Leach, 1980). Although the reduction of black scurf on daughter tubers would be a major goal of disease control, the assessments of the formation of black scurf on the seed pieces was useful, because it demonstrated whether or not potential antagonists were able to inhibit sclerotia formation. However, control of black scurf on the seed tuber may or may not accurately predict ability to reduce scurf on progeny tubers, because some organisms may not persist long enough to provide protection of developing tubers. Nevertheless, this method provides a relatively simple, effective way to assess black scurf formation on tubers while screening biocontrol or chemical treatments for suppression of stem canker on potato shoots.

Different formulations and rates of *L. arvalis*, *T. virens*, and *T. harzianum* were also compared for efficacy. There were no significant differences between rates or formulations of *T. virens* across all trials for black scurf. However, as expected, the formulation tended to be more consistent within the different trials for both stem canker and black scurf. Both the initial rate (3 g per pot) and reduced rate (1.5 g per pot) *of L. arvalis* ZH-1 reduced black scurf, but only the initial rate reduced stem canker. Although the spore suspension of *T. harzianum* reduced stem canker, surprisingly the commercial product was not very effective in the present study. In field studies with the same type of soil as the soil used in the present experiments, the commercial product did not control black scurf or stem canker (Larkin, 2001, 2002). Spore counts were conducted on the formulations to confirm viability. All counts were consistent with the spore counts provided on the label by the manufacturer. The commercial formulation may not have been successful because of the specific soil type used in these experiments, the other specific environmental conditions of the product, or perhaps it is not the best formulation for use with potato.

Although none of the biocontrol treatments provided control for stem canker and black scurf over all trials, many showed potential as effective antagonists against Rhizoctonia disease of potato. Additional studies on the mechanism(s) of action of newly discovered antagonists are necessary to fully understand the potential beneficial role they may serve in a sustainable potato cropping system. In addition, field experiments are needed, particularly in regard to season long control of black scurf. Variability seen with biocontrol may be best addressed by using combinations of successful antagonists or by applying beneficial organisms within effective crop rotations. These integrated management practices may potentially increase the consistency and efficacy of biocontrol.

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